Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v24i3.5297

ANALYSIS OF HAEMATOLOGICAL, SERUM, TISSUE BIOCHEMICAL PARAMETERS OF ETHANOLIC EXTRACTS OF AMARANTHUS SPINOSUS TREATED FEMALE ALBINO RATS.

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ABSTRACT
Amaranthus spinosus a important medicinal plant have various pharmacological properties and found throughout India. The antifertility activity of the ethanolic extract of *Amaranthus spinosus* (ASE) was seen earlier and the haematological parameters after treatment in rats were not altered. The serum biochemical parameters in ASE treated rats revealed the levels of albumin, creatinine, GGT, LDH, SGOT, SGPT, urea, uric acid, total bilirubin and total protein were not altered significantly. The cholesterol level was very high and the results were found statistically significant when compared to control group animals. The female hormone levels were altered significantly and the thyroid hormone levels were not altered. In the investigation of tissue biochemical parameters the cholesterol, estrogen and progesterone level altered and subsequently there was reduction in the weights of reproductive organs. Results of tissue biochemical parameters study in the reproductive organ of ASE treated rats altered the protein, glycogen, sialic acid, cholesterol, ascorbic acid, acid phosphatase and alkaline phosphatase. In conclusion the ASE treated female rats indicate adverse effect on uterine milieu, making it unsuitable for implantation.

KeyWords: *Amaranthus spinosus*, serum biochemical parameters, female hormone and tissue biochemical parameters.

INTRODUCTION
Fertility control is an issue of global and national public health concern. The current method of contraception results in unacceptable rate of unintended pregnancies. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. **(1)** In India medicinal plants have been screened for contraceptive potential and anti-fertility effects because of the health sector has always been concerned about the population explosion. So far herbal contraception has not reached the level of contraceptive protection as the pill, but it finds a path as alternatives for women who have difficulty with modern contraceptives.
Numerous herbs have been used as abortifacient, contraceptives and modern scientific research has confirmed anti-fertility effects in at least some of the herbs tested in male and female animal models activity and the active agents. **(2)** Many steroidal and non-steroidal compounds have been used as

contraceptive and antiovulatory agent to control fertility. Though they act as potent antifertility agents, they are not free from marked side effects.

Amaranthus extracts have been used in ancient Indian, Nepalese, Chinese and Thai medicine to treat several conditions including urinary infections, gynecological conditions, diarrhea, pain, respiratory disorders, diabetes and also as diuretic.**(3,4)** Hence, search for a new potent antifertility substance with minimal side effects are in progress. And there were no documented evidence referring to the anti-fertility effects of whole plants extracts of *Amaranthus spinosus* in animal studies. Thus, the present study was an attempt to investigate the effects of the whole plants of *Amaranthus spinosus* ethanolic extracts on the antifertility actions in female rats and to study the various blood related parameters. **(3,4)**

Collection of plant & Extraction procedure

The whole plants of *Amaranthus spinosus* from Kothagiri, Ooty district; Tamilnadu. Authentication was done by the Scientists, Botanical Survey of India, Agricultural University, Coimbatore- 641 003. After authentification fresh plant materials of *Amaranthus spinosus, was* collected in bulk. The collected plants were washed in, dried under shade and pulverized by mechanical grinder and sieved. The powdered material was successfully extracted with ethanol (70% v/v) by hot continuous percolation in Soxhlet apparatus, filtrated through Whattman filter paper No. 40, the filtrates were evaporated to dryness and were subjected for following studies.

Experimental animals:

Healthy albino Wistar rats (female) after approval from the Institutional Animal Ethical Committee, C.L.Baid Metha College of Pharmacy, Chennai, Tamil Nadu, (IAEC / II / 02 / CLBMCP / 2013 dated 21.01.2013) were used for the studies. Acclimatization, housing and feeding conditions were followed as per CPCSEA norms. The experiments were conducted on adult female young virgin Wistar rats between 8 and 12 weeks old after 2 weeks of acclimatization, the animals were randomly assigned for various experimental groups. Each group containing 6 animals were housed individually and were allowed free access to standard pellet diet and tap water *ad libitum*. They were maintained in controlled laboratory conditions of 12 hrs dark/light cycle, 22±2°C temperatures and 45-60% humidity.

Grouping of animals for estimation of haematological, serum and tissue biochemical activity of ASE (5)

Group I- served as control received Tween 80, 2% for7 days from day 1 to day 7; **Group II** – received ASE at 200 mg / kg for 7 days from day 1 to day 7. **Group III** – received ASE at 400 mg/ kg for 7 days from day 1 to day 7. **Group IV**- received ASE at 200 mg/ kg for 3 days from day 1 to day 3, which detects antizygotic activity. **Group V**- received ASE at 400 mg/ kg for 3 days from day 1 to day 3, which detects antizygotic activity. **Group VI** – received ASE at 200 mg/ kg for 2 days from day 1 to day 2, which detects blastocystotoxic activity. **Group VII**- received ASE at 400 mg/ kg for 2 days from day 1 to day 2, which detects blastocystotoxic activity. **Group VIII** – received ASE at 200 mg/ kg for 4 days from day 6 to day 9, which detects anti implantation or early abortifacient activity. **Group IX**- received ASE at 400 mg/ kg (p.o. daily) for 4 days from day 6 to day 9, which detects anti implantation or early abortifacient activity. All the groups were treated through gastric lavage per oral.

Table 1: Grouping of rats on different Phases of estrous cycles after treatment of ASE

Sl. No	Groups / Activity	Treatment
1	Control (1^{st} day to 10^{th} day)	Rats treated with tween 80 (2%) 1 ml/ kg/p.o suspension for 7 days.
2	Antiovulatory effect (1-7 days after mating)	Rats treated with ASE (200mg / kg/ p.o.) for 1- 7 days after mating.

3	Antiovulatory effect (1-7 days after mating)	Rats treated with ASE (400mg / kg/ p.o.) for 1- 7 days after mating.
4	Antizygotic activity (1-3 days after mating)	Rats treated with ASE (200mg / kg/ p.o.) for 1-3 days after mating.
5	Antizygotic activity (1-3 days after mating)	Rats treated with ASE (400mg / kg/ p.o.) for 1-3 days after mating.
6	Blastocidal activity (4^{th} and 5^{th} days after mating)	Rats treated with ASE (200mg / kg/ p.o.) for 4^{th} and 5^{th}day after mating.
7	Blastocidal activity (4^{th} and 5^{th} days after mating)	Rats treated with ASE (400mg / kg/ p.o.) for 4^{th} and 5^{th} day after mating.
8	Anti implantation activity (6^{th} and 7^{th} days after mating)	Rats treated with ASE (200mg / kg/ p.o.) for 6^{th} and 7^{th} day after mating.
9	Anti implantation activity (6^{th} and 7^{th} days after mating)	Rats treated with ASE (400mg / kg/ p.o.) for 6^{th} and 7^{th}day after mating.

Investigation of Haematological/ Serum bio chemical parameters (5)

After the study of ASE treatment was given blood samples were collected and divided into part-I and part-II. To one part of the blood in test tube, two drops of sodium citrate (3.8%w/v) was added and preceded for haematological parameters. Another part of the blood in the test tube was allowed to clot at room temperature, centrifuged at 4500 rpm for 15 min; the pure serum was collected from the supernatant and it was subjected to biochemical analysis in Secomam semi auto analyzer which consists of the following technical characteristics.Values are mean ± SEM; n=6 in each group; ASE treated rats were compared with control group rats the values statistically (*= $P<0.05$ significant;**= $P<0.01$ moderately significant, *= $P<0.001$ highly significant).

Estimation of Haematological parameters

The following estimation of White blood corpuscle, Red blood corpuscle, Hemoglobin, Haematocrit, Mean corpuscular volume, Mean corpuscular haemoglobin, Mean corpuscular haemoglobin concentration were performed.**(6)**

Estimation of serum bio chemical parameters in rats

The following estimation of Albumin, Alkaline phosphatase and Creatinine by Tietz N. *et al.,* (7); Cholesterol by Allain C. *et al.,* **(8)**; Glucose by VH B, Ajay SS, GGT **(9)**, LDH by HU Bergmeyer, **(10)**; Glutamic Oxaloacetic Transaminase & Glutamic Pyruvic Transaminase by Mohanty S, *et al.*, **(11)**; Urea by Wheatherburn M.W, **(12)** ; Uric Acid & uric acid by Trivedi R.C. *et al.,* 1978 **(13)** ; Bilirubin by Defreese JD. *et al.,* and Total protein by Tietz NW, **(7)** and Triglycerides by Abdel-Rahman Z.., **(15)**

Estimation of serum hormonal assay:

The following estimation of Oestradiol by Tsang *et al.,* **(16)**; Progesterone by Scholler R *et al.,* and T3, T4 and TSH by Inada MK.**(18)**

Estimation of tissue biochemical parameters

Ovary and uterus were washed with chilled isotonic saline and the tissue homogenates were prepared in ice cold 0.1 M Tris-HCl buffer (pH 7.2) and used for the assay of clinical marker enzymes. The following parameters were studied in ovary and uterus of rats. Protein by Scopes RK

et al., **(19)**; Glycogen by Chun Y, Yin ZD. **(20)**; Sialic acid by Sugahara K, **(21)** ; Cholesterol by Nauck M *et al;* **(22)**; Ascorbic acid by Washko PW, *et al.*, (23) ; Acid phosphatise and Alkaline phosphatase by Oser, **(24)**.

Results and discussion

Table-7.19: Results of the hematological parameters of ASE treated rats

Groups	WBC (thous/mcl)	RBC (mill/mcl)	Hb (g/dl)	HT (%)	MCV (fl)	MCH (pg)	MCHC (%)	Lymphocytes (%)	Monocytes (%)	Heterophils (%)
i- Control	8.88±2.47	8.65±2.24	13.74±1.72	39.04±1.04	54.24±2.48	20.22±1.12	41.34±2.37	42±1.67	3±0.27	44±1.24
ii- Antiovulatory effect	8.64±2.69	8.54±1.68	13.44±2.42	40.41±2.34	56.68±2.46	21.22±2.62	42.33±0.33	42±1.76	3±0.4	43±2.40
iii - Antiovulatory effect	8.52±3.6	8.11±1.09	14.48±2.11	41.27±2.25	55.75±2.67	21.22±1.98	42.43±0.93	41±1.88	3±0.1	43±2.30
iv - Antizygotic activity	8.29±2.36	8.17±1.73	13.18±2.51	41.32±1.38	53.64±2.33	21.85±1.66	41.24±2.34	44±2.55	3±0.2	45±2.30
v- Antizygotic activity	8.09±2.61	7.78±1.47	13.45±1.55	39.38±1.48	52.47±2.77	21.24±1.94	42.45±2.36	42±2.57	3±0.4	44±2.60
vi- Blastocidal activity	8.09±1.85	8.48±1.41	13.58±1.52	41.24±1.56	54.34±2.11	21.24±1.84	43.44±2.33	43±2.77	3±0.7	42±2.42
vii- Blastocidal activity	8.66±2.56	8.51±1.87	14.24±1.26	42.42±1.26	55.11±2.24	21.07±2.78	42.36±2.35	42±2.67	3±0.8	42±2.60
viii- Anti implantation activity	8.15±3.6	8.17±2.11	13.28±1.27	41.28±2.28	54.28±2.38	21.16±1.96	42.24±2.22	43±2.72	3±0.9	42±1.32
ix-Anti implantation activity	8.58±1.25	8.17±2.11	13.55±1.37	42.55±3.5	54.11± 0.36	22.43±1.14	42.52±2.92	42±2.26	3±0.6	43±2.36

Table-7.23: Results of the biochemical parameters in ASE treated rats

Groups	Albumin (g/dl)	ALP (U/l)	Creatinine (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)	GGT (U/l)	LDH (U/l)
i- Control	3.71±0.5	86.24±2.4	0.6±0.05	72.04±0.14	90.24±3.47	90.24±3.47	136.45±5.8
ii- Antiovulatory effect	3.11±0.7	70.11±2.1*	0.6±0.04	140.64±4.41***	71.24±1.47*	91.01±1.42	130.08±6.4
iii - Antiovulatory effect	3.17±0.5	69.14±1.4*	0.7±0.01	144.78±3.41***	70.33±2.12*	92.41±4.14	136.09±4.1
iv - Antizygotic activity	3.21±0.6	69.78±3.1*	0.7±0.12	142.45±2.15***	73.67±1.64*	95.01±3.74	135.04±5.8
v- Antizygotic activity	3.24±0.3	71.25±2.3*	0.6±0.21	147.12±2.47***	74.54±2.21*	97.11±1.84	139.37±9.6
vi- Blastocidal activity	3.25±0.4	71.28±0.4*	0.6±0.24	141.27±4.78***	72.55±4.96*	96.05±1.+4	136.28±4.7
vii- Blastocidal activity	3.26±0.1	69.54±0.9*	0.7±0.34	148.54±2.49***	70.34±2.17*	94.08±1.82	125.37±7.2
viii- Anti implantation activity	3.24±0.4	71.26±2.6*	0.6±0.12	147.78±2.47***	71.24±2.51*	93.27±4.57	129.98±1.3
ix-Anti implantation activity	3.32±0.3	70.55±2.6*	0.6±0.22	149.89±4.49***	75.44±1.21*	94.06±2.26	139.97±3.7

Table-7.24: Results of the biochemical parameters in ASE treated rats

Groups	SGOT (U/l)	SGPT (U/l)	Urea (mg/dl)	Uric acid (mg/dl)	Total Bilirubin (mg/dl)	Total Protein (g/dl)	Triglycerides (mg/dl)
i- Control	68.24±4.2	26.14±1.74	21.54±1.13	5.24±0.44	0.62±0.05	6.24±0.98	134.72±4.4
ii- Antiovulatory effect	66.25±1.7	28.33±3.78	22.36±1.17	6.01±0.09	0.68±0.01	7.11±0.14	136.25±1.7
iii - Antiovulatory effect	64.85±0.9	29.54±2.55	22.45±1.73	5.98±0.11	0.64±0.01	7.12±0.21	135.69±2.5
iv - Antizygotic activity	68.65±2.8	28.11±3.32	22.74±1.05	5.12±0.17	0.62±0.01	6.88±0.21	137.14±3.4
v- Antizygotic activity	69.63±3.5	26.18±1.74	23.58±1.77	4.98±0.16	0.63±0.02	7.04±0.92	141.45±2.5
vi- Blastocidal activity	65.85±6.7	28.97±1.42	24.11±1.0 3	4.66±0.17	0.64±0.03	6.85±0.22	142.65±3.4
vii- Blastocidal activity	66.35±5.8	25.07±1.25	24.65±0.13	4.98±0.21	0.69±0.03	6.58±0.24	145.41±1.8
viii- Anti implantation activity	69.65±4.9	27.04±1.14	24.85±1.35	5.04±0.14	0.25±0.02	7.04±0.26	136.23±1.7
ix-Anti implantation activity	65.66±5.3	28.57±3.74	22.14±1.44	4.89±0.15	0.64±0.01	6.98±0.12	138.47±4.4

Table-7.31: Results of tissue weight analysis in ASE treated rats

Groups	Liver(g)	Heart (g)	Kidney(g)	Uterus(mg)	Vagina(mg)
i- Control	3.07±0.09	0.987±0.03	3.07±0.09	255±0.019	211±0.052
ii- Antiovulatory effect	3.31±0.07	0.988±0.05	3.14±0.07	284±0.016*	255±0.035*
iii - Antiovulatory effect	3.14±0.12	0.997±0.02	3.24±0.04	288±0.026*	254±0.056*
iv - Antizygotic activity	3.44±0.04	0.957±0.07	3.14±0.01	287±0.013*	259±0.024*
v- Antizygotic activity	3.33±0.05	0.975±0.05	3.16±0.08	286±0.018*	261±0.045*
vi- Blastocidal activity	3.24±0.07	0.952±0.06	3.15±0.05	281±0.015*	260±0.027*
vii- Blastocidal activity	3.21±0.05	0.975±0.04	3.14±0.02	289±0.032*	266±0.035*
viii- Anti implantation activity	3.11±0.6	0.966±0.03	3.21±0.09	282±0.014*	249±0.043*
ix-Anti implantation activity	3.21±0.04	0.977±0.07	3.14±0.6	291±0.017*	254±0.017*

Table-7.28: Results of serum hormonal assay of ASE treated rats

Groups	Oestradiol (Pg/ml)	Progesterone (Pg/ml)	Triiodothyronine (µU/ml)	Thyroxin (µU/ml)	Thyroid stimulating hormone (µU/ml)
i- Control	69.37±2.15	13.13±0.94	0.33 ± 0.11	1.28 ± 0.12	65.11 ± 1.19
ii- Antiovulatory effect	32.44±1.41**	09.11±0.49**	0.32 ± 0.14	1.18 ± 0.26	68.21 ± 1.55
iii - Antiovulatory effect	32.19±2.52**	09.74±0.47**	0.31 ± 0.14	1.47 ± 0.29	64.33 ± 1.35
iv - Antizygotic activity	31.75±2.23**	09.34±0.74**	0.33 ± 0.54	1.24 ± 0.23	63.11 ± 1.11
v- Antizygotic activity	33.35±2.02**	09.49±0.17**	0.33 ± 0.47	1.33 ± 0.24	66.01 ± 1.22
vi- Blastocidal activity	30.46±2.58**	09.25±0.18**	0.34 ± 0.87	1.22 ± 0.29	69.14 ± 1.25
vii- Blastocidal activity	30.24±2.26**	09.97±0.19**	0.31 ± 0.11	1.16 ± 0.16	67.11 ± 1.78
viii- Anti implantation activity	30.54±2.41**	09.44±0.24**	0.31 ± 0.31	1.84 ± 0.11	69.26 ± 1.55
ix-Anti implantation activity	31.11±2.11**	09.41±0.21**	0.33 ± 0.24	1.64 ± 0.24	69.12 ± 1.25

Table -7.37: Results of tissue biochemical parameters study in the reproductive organ (ovary) of ASE treated rats

Groups	Protein (mg/g)	Glycogen (mg/g)	Sialic acid (mg/g)	Cholesterol (mg/g)	Ascorbic acid (mg/g)	Acid Phosphatase (mgpi/g/h)	Alkaline phosphatase (mgpi/g/h)

i- Control	166.22±3.8	8.69± 0.23	0.964 ± 0.01	4.18± 0.15	14.45 ± 0.64	5.87 ± 0.21	6.21 ± 0.23
ii- Antiovulatory effect	165.11±2.7	4.04± 0.11***	0.486± 0.03*	10.52± 0.21***	7.11± 0.09*	2.11± 0.051**	3.14± 0.12**
iii - Antiovulatory effect	163.85±3.2	4.11±0.31***	0.521± 0.01*	11.66± 0.19***	7.12± 0.08*	2.14± 0.04**	3.45± 0.13**
iv - Antizygotic activity	168.22±2.8	4.21± 0.01***	0.498± 0.02*	12.46± 0.17***	7.04± 0.11*	2.42± 0.03**	3.45± 0.04**
v- Antizygotic activity	168.37±1.2	4.33± 0.12***	0.548± 0.03*	12.31± 0.12***	7.07± 0.08*	2.25± 0.074**	3.79± 0.15**
vi- Blastocidal activity	161.11±2.3	4.29± 0.02***	0.522± 0.01*	12.22± 0.13***	7.15± 0.11*	2.35± 0.09**	2.96± 0.13**
vii- Blastocidal activity	169.82±3.8	4.17± 0.04***	0.531± 0.02*	11.25± 0.14***	7.31± 0.15*	2.25± 0.21**	3.34± 0.13**
viii- Anti implantation activity	165.28±2.8	4.26± 0.05***	0.514± 0.02*	11.58± 0.16***	7.12± 0.05*	2.22± 0.18**	3.57± 0.12**
ix-Anti implantation activity	170.19±2.1	4.62± 0.14***	0.584± 0.01*	13.45± 0.13***	7.12± 0.09*	2.29± 0.04**	2.44± 0.12**

Table -7.40: Results of tissue biochemical parameters study in the reproductive organ (uterus) of ASE treated rats

Groups	**Protein (mg/g)**	**Glycogen (mg/g)**	**Sialic acid (mg/g)**	**Cholesterol (mg/g)**	**Ascorbic acid (mg/g)**	**Acid Phosphatase (mgpi/g/h)**	**Alkaline phosphatase (mgpi/g/h)**
i- Control	155.11±2.4	7.69± 0.23	0.954 ± 0.01	5.18± 0.15	13.35 ± 0.37	6.07 ± 0.17	6.37 ± 0.42
ii- Antiovulatory effect	154.11±2.2	4.14± 0.12***	0.498± 0.02*	9.89± 0.22***	7.98± 0.07*	2.44± 0.02**	3.12± 0.11**
iii - Antiovulatory effect	158.85±3.1	4.17±0.31***	0.514± 0.02*	9.89± 0.12***	7.65± 0.04*	2.25± 0.08**	3.42± 0.11**
iv - Antizygotic activity	159.29±2.1	4.25± 0.03***	0.488± 0.02*	9.98± 0.13***	7.58± 0.11*	2.35± 0.09**	3.43± 0.04**
v- Antizygotic activity	155.35±1.8	4.36± 0.14***	0.501± 0.02*	9.99± 0.11***	7.78± 0.02*	2.66± 0.05**	3.49± 0.15**
vi- Blastocidal activity	154.17±2.2	4.21± 0.05***	0.506± 0.01*	10.98± 0.11***	7.55± 0.14*	2.26± 0.08**	2.97± 0.11**
vii- Blastocidal activity	159.81±3.1	4.17± 0.04***	0.504± 0.04*	11.11± 0.11***	7.68± 0.12*	2.36± 0.29**	3.07± 0.11**
viii- Anti implantation activity	157.18±2.2	4.28± 0.01***	0.507± 0.03*	11.04± 0.06***	7.47± 0.059*	2.55± 0.16**	3.38± 0.12**
ix-Anti implantation activity	158.49±2.6	4.67± 0.11***	0.511± 0.02*	11.01± 0.03***	7.37± 0.19*	2.11± 0.03	2.98± 0.12**

Discussion

The hematological parameters of ASE 200 and 400 mg/kg/po treated rats for estimation of WBC & RBC counts, haemoglobin, MCV, MCH, MCHC, HT, lymphocytes, monocytes and heterophils were not altered significantly when compared to control group animals proving the safety of ASE. Further WBC counts were also not altered. The serum albumin level responsible maintaining and regulating the colloidal osmotic pressure of blood was not significantly altered indicating that ASE does not have any adverse effect in the treated rats. Alkaline phosphatase is associated with the decidual cell reaction and play important role in implantation and a significant ($P<0.05$) decline in uterine acid and alkaline phosphatase activity in extracts treated mated female rats indicated an adverse effect on uterine milieu and thus making it unsuitable for implantation.**(25)**

Administration of 200 & 400mg/kg/p.o. of ASE in rats the serum creatinine level was not significantly altered. Cholesterol is needed as a structural element in all cell membranes, and is a building block for some hormones and other essential body functions ASE treatment the serum Cholesterol level was found to be significantly high ($P<0.001$). Glucose is the primary energy source for all animals and the human body a decrease in the circulating glucose content in ASE treated female rat indicates poor nutritive support to the developing blastocyst for their survival and hence the antifertility effects were seen. Further on the administration of ASE, in rats the serum GGT, LDH, SGOT, SGPT, urea, uric acid, Bilirubin, total protein, serum triglycerides level was not altered. **(26-27)**

Estrogen is a steroid synthesized from both tissue and circulating cholesterol. During administration of ASE at 200 & 400mg/kg b.wt./day in rats the oestradiol level was decreased in the circulating blood in extract treated female rats which indicates poor support to the developing embryo for their survival resulting in antifertility effect. Progesterone is a steroid synthesized from both tissue and circulating cholesterol. ASE treatment in rats level of progesterone was decreased indicates poor support to the developing embryo for their survival and hence the antifertility effect is observed.**(28)** ASE treatment the T3, T4 and TSH level was not altered which may be due to short period of administration of test drugs and the differentiation was found to be insignificant.
Administration of ASE treated rats the tissue weight of liver, heart, kidney were not altered significantly; uterus and vaginal weights were altered significantly when compared to control group of animals. The decrease in estrogen and progesterone parameters of female reproductive organs and insufficient level of circulating estrogen and progesterone which was essential for maintenance of their physiological integrity and due to this uterus and vagina weights were decreased. **(29)**
In the present study, a significant decline ($p<0.001$) in the uterine glycogen content in ASE treated rats indicated poor nutritive support to the developing blastocyst for their survival which can account for their antifertility action. Sialomuco protein, a derivative of sialic acid, forms mucous in the ovary and uterus which sticks around the blastocyst fluid and helps in the attachment of the blastocyst, a significant decrease ($p<0.05$) in the sialic acid ASE treated female rats was observed. **(30)** Cholesterol is the precursor of sex hormones and is utilized during steroidogenesis cholesterol concentration of ovary and uterus was increased after ASE treatment, indicating non utilization of cholesterol by the system. Ascorbic acid anti-oxidant, anti-inflammatory, anti-viral agent and an immune stimulant ascorbic acid can restore the ciliary mucus cells. In the present study, ovary and uterus ascorbic acid levels were decreased after ASE treatment. Alkaline and acid phosphatases are associated with the decidual cell reaction and implantation. A significant decline in ovary and uterus alkaline and acid phosphatase activity in ASE treated mated female rats indicate adverse effect on uterine milieu, making it unsuitable for implantation.**(31)**

Conclusion:
In a nut shell, on administration of ethanolic extracts of *Amaranthus spinosus*, at the dose 200 & 400mg/kg b.wt./day altered the serum, tissue biochemical parameters and not altered haematological parameters. In conclusion the ASE treated female rats indicate adverse effect on uterine milieu, making it unsuitable for implantation.

Ethical approval: IAEC, C.L.Baid Metha College of Pharmacy, Chennai, Tamil Nadu, (IAEC / II / 02 / CLBMCP / 2013 dated 21.01.2013).

Funding source: None.

Conflicts of interest: None declared.

Acknowledgement: The author wish to thank Mr. .M. Gurumani, who has helped in the research.

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